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PTO/SB/21 (09-04)
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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 39

Application Number	10/665,297
Filing Date	09/22/2003
First Named Inventor	William F. Long
Art Unit	2881
Examiner Name	Kara E. GEISEL
Attorney Docket Number	15186-32US

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
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- ☐ Express Abandonment Request
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- ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
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- ☐ Status Letter
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Letter; Return Postcard

Remarks

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CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

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OGILVY
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February 8, 2005

Mail Stop Patent Application

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
U.S.A.

Sir:

Re: United States Patent Application No. 10/665,297

Filed:	**09/22/2003**
Title:	**METHOD AND APPARATUS FOR TIME RESOLVED OPTICAL IMAGING OF BIOLOGICAL TISSUES AS PART OF ANIMALS**
Inventor(s):	**William F. Long et al.**
Assignee:	**ART ADVANCED RESEARCH TECHNOLOGIES INC.; INSTITUT NATIONAL D'OPTIQUE**
Our File:	**15186-32US**

Enclosed herewith for filing against the above-identified application, is the required certified copy of the priority application in this matter, i.e. International Patent Application No. PCT/IB02/04698, as filed November 11, 2002.

Respectfully submitted,

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Encls.
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TRAITÉ DE COOPÉRATION EN MATIÈRE DE BREVETS (PCT)

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International Application No. / Demande internationale n° **PCT/IB02/04698**

International Filing Date / Date du dépôt international **11 November 2002 (11.11.02)**

Geneva/Genève,

21 November 2002 (21.11.02)

International Bureau of the World Intellectual Property Organization (WIPO)

Bureau International de l'Organisation Mondiale de la Propriété Intellectuelle (OMPI)

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Head, PCT Receiving Office Section
Chef de la section "office récepteur du PCT"

15186-32PCTP

PCT REQUEST

Original (for SUBMISSION) - printed on 10.11.2002 10:19:42 PM

0	For receiving Office use only	
0-1	International Application No.	PCT / IB 0 2 / 0 4 6 9 8
0-2	International Filing Date	11 NOVEMBER 2002 (11. 11. 02)
0-3	Name of receiving Office and "PCT International Application"	INTERNATIONAL BUREAU OF WIPO PCT International Application
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.10.2002)
0-5	Petition	
	The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	International Bureau of the World Intellectual Property Organization (RO/IB)
0-7	Applicant's or agent's file reference	15186-32PCTP
I	Title of invention	METHOD AND APPARATUS FOR TIME RESOLVED OPTICAL IMAGING OF BIOLOGICAL TISSUE
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PCT REQUEST 15186-32PCTP

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PCT REQUEST 15186-32PCTP

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V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE BG CH&LI CY CZ DE DK EE ES FI FR GB GR IE IT LU MC NL PT SE SK TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	Exclusion(s) from precautionary designations	NONE
VI	Priority claim	NONE
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)

PCT REQUEST 15186-32PCTP

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VIII	Declarations	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	6	-
IX-2	Description	18	-
IX-3	Claims	~~6~~ 4	-
IX-4	Abstract	1	EZABST00.TXT
IX-5	Drawings	5	-
IX-7	TOTAL	~~35~~ 36	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-17	PCT-EASY diskette	-	Diskette
IX-19	Figure of the drawings which should accompany the abstract	2	
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative		
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X-1-2	Name of signatory	ANGLEHART, James	
X-1-3	Capacity	Patent Agent and Partner of the firm	

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	11 NOVEMBER 2002 (11.11.02)
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP

PCT REQUEST

15166-32PCTP

Original (for SUBMISSION) - printed on 10.11.2002 10:18:42 PM

10-6	Transmittal of search copy delayed until search fee is paid	

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11-1	Date of receipt of the record copy by the International Bureau	

METHOD AND APPARATUS FOR TIME RESOLVED OPTICAL IMAGING OF BIOLOGICAL TISSUE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This is the first application filed for the present invention.

TECHNICAL FIELD

[0002] The invention relates to the field of optical imaging of turbid media such as biological tissues. More specifically, the invention relates to time resolved optical data acquisition for use in optical imaging.

BACKGROUND OF THE INVENTION

[0003] Different types of imaging techniques such as positron emission tomography (PET), magnetic resonance imaging (MRI) and ultrasound imaging are available that can non-invasively gather information from within biological tissues as a basis for image reconstruction. More recently, another imaging technique, namely optical imaging has been the subject of intense research and commercial development.

[0004] Optical imaging is based on the information that can be derived from the analysis of the signal resulting from the interaction of light with matter as it is propagated within an object. Optical images can be reconstructed using three different approaches namely continuous wave (CW), time domain (TD) and frequency domain (FD). CW is the simplest and least expensive of the three techniques but provides only limited information with regards to the spatial distribution of internal optical properties of the object being imaged. TD and FD, by conveying

information on the time required by photons to travel within the object (FD through the Fourier transform), can be used to calculate the spatial distribution of optical characteristics of the object, such as absorption and scatter coefficients, *via* well known photon diffusion equations (Hawrysz and Sevick-Muraca, Neoplasia, vol.2 No 5, pp388-417, 2000). This spatial information can then serve to reconstruct optical images.

[0005] Optical imaging is particularly attractive in view of its non-invasiveness which permits the acquisition of *in vivo* information without damaging biological tissues. Furthermore the technique may be useful to monitor drug distribution, detect the presence of abnormalities within organs, or map physiological activities within mammals.

[0006] However, widespread utilization of optical imaging systems has been impeded by some undesirable characteristics of existing systems. For example, optical imaging devices often require cumbersome arrangements of optical fibers that are used to transport the light to and from the object. Such systems have been described for example by Ntziachristos and Weissleder in patent application WO 02/41769 and by Hillman et al. Phys. Med. Biol, 46 (2001)1117-1130. The type of arrangement for the optic components described in these references requires a time consuming alignment of the region of interest with the optic fibers used to illuminate the object and detect the optical signal. This type of arrangement is particularly problematic when imaging is performed on living tissues of mammals.

[0007] Ease of data acquisition and in particular ease of the positioning the object relative to the optic

components is especially important in applications requiring high throughput such as in clinical settings or in research that make use of small mammals such as mice. In this respect, commercially available optical imaging systems for imaging small mammals have been developed. For example, a bioluminescence imaging system developed by Xenogen Corp. (Biophotonics, vol.9, No.7 pp48-51, 2002) has been designed to collect light emanating from small mammals. However, this imaging device suffers from certain disadvantages. For example, it requires the presence of autofluorescent molecules that need to be injected in the animal and which have a spatially restricted biodistribution profile therefore greatly reducing the flexibility in imaging desired region of interests (ROI). Furthermore, the technique is limited by the number of luminescent molecules that are currently available. And, importantly, the system does not allow time resolved data to be acquired.

[0008] In view of the above, it would be desirable to provide an optical imaging system for imaging turbid media such as biological tissues that allows time resolved optical data to be acquired with increased flexibility and efficiency.

SUMMARY OF THE INVENTION

[0009] The invention relates to the field of optical imaging of turbid medium such as biological tissues. More specifically, the invention relates to time resolved optical data acquisition for use in optical imaging.

[0010] In a broad aspect of the invention there is provided a system and method for time resolved optical imaging of biological tissue. The design of the optical components of the present system allows a beam of light to be directionally propagated through air, that is to say through free-space optics, to impinge on desired points of illuminations in a region of interest (ROI) of the tissue. Light re-emitted from the tissue is collected at collection points and directionally propagated through air (i.e. through free-space optics) towards a detector. The fact that the light is propagated through air allows for a greater flexibility in scanning different ROI by eliminating the need for cumbersome fiber optics arrangements. Thus there is no need for directly contacting the tissue with optical components thereby leaving sufficient space to manipulate or access the mammal.

[0011] The optical design of the system permits illumination and light collection through air of nearby points in the ROI with minimal interference between the illumination beam, or the light reflected at the skin/air interface, and the collected light.

[0012] In one embodiment, there is provided a time resolved optical imaging method which comprises illuminating, at one or more wavelengths, a region of interest of a mammal at a plurality of predetermined illumination points using a pulsed light beam. The beam is propagated through air and directed by using appropriate optical components to the illumination points. A plurality of predetermined collection points are collected by optic components having a configuration enabling selective collection of light from well defined surface areas in the ROI. The

collected light is then directed to a detector to produce an optical signal that can be used to generate an optical image using well known reconstruction algorithms.

[0013] In a further aspect of the invention there is provided a system for collecting optical data for use in time resolved imaging comprising one or more light sources for providing a light beam at one or more wavelengths, illuminating optic components for directionally propagating the beam through air such that a region of interest of the biological tissue is illuminated at a plurality of illumination points thereby injecting light into the tissue, collecting optic components for collecting light re-emitted at a plurality of predetermined collection points in the region of interest and for directionally propagating through air the collected light and a time correlated detector for detecting the collected light.

[0014] The system of the invention may advantageously be configured to acquire data for topographic or tomographic imaging. Topographic imaging is achieved by maintaining constant the distance between collection points and illumination points. Synchronized mirrors galvanometer are provided in the illumination and collection optics to achieve a constant distance between the illumination points and the detection points.

[0015] Tomographic imaging requires that light re-emitted from the animal be sampled at several different collection points for each illumination point. The system of the present invention advantageously provides moveable mirrors in the illumination and collection optics that are independently controllable thereby providing means to

achieve a plurality of illumination points/ collection points configurations.

[0016] In another aspect there is provided a method and system for optical imaging of biological tissue containing fluorescent molecules. The tissue can be illuminated at an excitation wavelength while light re-emitted can be collected and detected at an emission wavelength. The system also enables detection of both the emission wavelength and the excitation wavelength.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Further features and advantages of the present invention will become apparent from the following detailed description, taken in combination with the appended drawings, in which;

[0018] FIG. 1 is a flow chart diagram of an embodiment of the method of the instant invention;

[0019] FIG. 2 is perspective view of an embodiment of the system of the invention;

[0020] FIG.3 is as schematic representation of an embodiment of the system in which the source comprises a plurality of lasers;

[0021] FIG. 4 schematically illustrates a raster scan pattern of illumination in a region of interest at the surface of a mammal;

[0022] FIG. 5 a schematic representation of an embodiment of the system of the invention in which the optical

components are mounted on a gantry to be rotated around the mammal to acquire data for tomographic imaging;

[0023] FIG. 6 is a schematic representation of an embodiment of the system of the invention in which the the optical components are fixed and the mammal is rotated to acquire data for tomographic imaging.

[0024] It will be noted that throughout the appended drawings, like features are identified by like reference numerals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0025] The invention relates to the field of optical imaging of turbid media such as biological tissues. More specifically the invention relates to time resolved optical data acquisition for use in optical imaging.

[0026] With reference to FIG.1 an embodiment of the method of the present invention for collecting optical data for use in time resolved optical imaging is generally described. At 2 pulsed light from a source of a selected intensity is directionally propagated in air (i.e through free space optics) to illuminate a plurality of predetermined illumination points in a ROI of a biological tissue. The light emanating from a plurality of collection points after diffusion through the tissue is selectively collected through free space optics at 4 and directionally propagated through free space at 6 towards a detector. The collected light is finally measured at 8 using the detector to produce a time resolved optical signal. Light emanating from points other than that being sampled is optically excluded from detection.

[0027] The embodiments of the system used for collecting the optical data will now be described referring to small mammals as the object to be imaged but it will be appreciated that a wide variety of biological tissues may be amenable to optical imaging using the technique described herein. These can be but are not limited to breast tissue, brain, tumors and the like.

[0028] A general schematic representation of the system of the present invention used for imaging small mammals is shown in FIG. 2. The system comprises a light source 10 capable of generating a beam of light 12 at one or more wavelengths, illuminating optics for directionally propagating the beam of light through air, i.e. through free space optics, to desired illumination points on the surface of the mammal 14, collecting optics for collecting the light 16 re-emitted from the mammal for directionally propagating the collected light to a detector 18, a moveable mammal supporting tray 20 mounted on a translational stage 22 and a computer 19 for controlling the source, the optics, the detector and the tray.

[0029] The illuminating optics comprises a moveable reflective mirror 24 which is preferably a mirror galvanometer. The beam is reflected by the mirror galvanometer at an angle θ and directed towards a thin angled mirror 26 which reflects the beam in a direction substantially perpendicular to the surface of the mammal being scanned. It can be appreciated that the partial rotation of the mirror galvanometer will modify the angle θ and direct the beam to a different point on the thin angled mirror and, consequently, to a different illumination point on the surface of the mammal.

Successive partial rotations of the mirror galvanometer 24 thus produces a line scan substantially parallel to the thin angled mirror. Lens 28 is optionally provided and positioned between the mirror galvanometer and the thin angled mirror such that the mirror galvanometer is at the focal distance of the lens to provide telecentric imaging. Filters such as neutral density filter 29 may also be positioned between the source and mirror galvanometer 24 to adjust the intensity of the light beam.

[0030] In an aspect of the invention, the light source is preferably a variable intensity laser emitting light at a specific wavelength. To produce a multi-wavelengths illumination a collection of lasers 31, such as diode lasers, each emitting light at different wavelengths may be used (Fig. 3). Appropriate filters such as filters 33 and 35 may be positioned between the source and the mammal and between the re-emitted light and the detector for the selection of wavelengths or to adjust the intensity of the light. A switching or dichroic mirror system may be used for either sequential or simultaneous illumination of the mammal at different wavelengths. Alternatively, a unique multi-wavelengths source of light may also be used. In the latter case, ranges of wavelengths or specific wavelengths may be selected by using filters, gratings or the like, as is well known by persons skilled in the art.

[0031] The preferred intensity of the source is determined in part by the amount of light energy that can be absorbed by the tissue without causing any significant damage to the tissue. For example, a laser emitting in the near infrared (NIR) should preferably be adjusted at

a power of about 200 mW or less for non-invasive imaging of biological tissues such as that comprising humans and small mammals.

[0032] Tray 20 supports the mammal while it is being imaged. The mammal is preferably anesthetized for the duration of the data collection to reduce movements to a minimum. Optionally the tray can be heated to maintain the body temperature of the mammal. Furthermore the tray can be displaced longitudinally on a translational stage 22 to position the mammal such that a plurality of line scans parallel to each other can be generated. This stepwise process is repeated a selected number of times to produce a raster scan of a region of interest (ROI). The raster scan can alternatively be achieved by longitudinally displacing the thin angled mirror 26. Figure 3 illustrates an example of a raster scan pattern at the surface of a mammal. The user defined ROI 40 delimits the area to be scanned which comprises the predetermined illumination points 42. The arrangement of the optic components also permits other scanning patterns to be performed.

[0033] Light re-emitted from the mammal is collected by the collecting optics which comprise collecting lens 34, reflective mirror 36 which is preferably a mirror galvanometer and lens 38. Collecting lens 34 is located above the ROI and above the thin angled mirror. The angular position of the mirror 36 relative to the incoming light and the detector determines which collection point is being sampled since only part of the light (corresponding to a given collection point) impinging on the mirror is reflected at the proper angle to reach the detector. Selective detection of the light

from a given collection point may be further enhanced by optically coupling the mirror galvanometer with lenses and/or pinholes.

[0034] The overall arrangement of the optics, which permits propagation of the light through air, allows for easy positioning and manipulation of the animal. Furthermore, since the system does not rely on optic fibers, a plurality of ROIs can be scanned without manipulating the animal simply by moving the tray so that a new ROI is brought in the focus of the optics. The movements of the tray can be controlled externally using the computer.

[0035] Upon impinging on the surface of the mammal, part of the light penetrates the skin and part is reflected at the air/skin boundary. The photons that are propagated within the mammal are absorbed and scattered, thereby producing a large number of photon paths. In biological tissues absorption may arise as a result of the presence of natural (endogenous) or exogenous chromophores while scattering is triggered by the presence of macromolecular structures such as proteins, lipids and the like which create refractive index inhomogeneities. The fraction of the light that is not absorbed ultimately exits the mammal by diffusing through the skin barrier at various distances from the illumination point. It can be appreciated that photons that have traveled deeper in the tissue will take a longer time to exit at the surface of the mammal. This provides the basis for the time resolved detection of the optical signal from which useful information about the optical properties of a region of interest can be extracted to be incorporated into image reconstruction algorithms. In optically homogeneous media the distance between the illumination point and the point

at which given photons exit is related to the effective depth of the average path of the photons. Thus the greater the distance between the points the greater the depth. While biological tissues are not optically homogeneous the distance between illumination points and the point of photon exit can also be considered to be related to the depth of the average path of photons.

[0036] In a preferred embodiment the time resolved method used in the system of the present invention is time domain (TD). In TD measurements the source is briefly pulsed and the optical signal is detected as a function of time to generate a time point spread function (TPSF). The source is preferably a laser source capable of generating pulses characterized by a width in the picoseconds range. Time correlated detectors such as time gated charged coupled devices (CCDs) and intensified CCDs (ICCDs), time correlated single photon counting devices(TCSPCDs), ultrafast semiconductor detectors (avalanche and PIN photodiodes), photomultipliers and streak cameras can be used. In a preferred embodiment a TCSPCD is used in the system of the present invention. TCSPCDs are capable of measuring the time taken by a photon to reach the detector as it travels through the illuminating optic, the tissue and the collecting optic. Time measurement is provided by a clock" circuitry electronically coupling the source and the detector. Such circuits are well known in the art. TCSPCD are very sensitive and advantageously allows the use of low power sources to minimize damage to the tissue being scanned.

[0037] In an embodiment of the invention, attenuation measurements similar to measurements obtained using

continuous wave can be generated using the system and method of the present invention by integrating the TPSF.

[0038] TCSPCDs are typically characterized by a time resolution in the picoseconds range. That is to say, the detector system can only distinguish between two incoming photons if their arrival at the detector is separated by at least the resolution limit of the detector. If two or more photons arrive within a time period equal to or smaller than the resolution limit, then only a single "event" is recorded by the detector. Thus, a TPSF generated using TCSPCD can be considered to be constituted of a plurality of time "bins" of a width equals to the resolution of the detector. Since a given pulse can only generate one "event" per time bin, a plurality of pulses are provided at each illumination point. The time bins of the TPSF are populated by the number "events" produced by the totality of the pulses injected in the tissue.

[0039] Statistically, the efficiency of detection, that is to say the ratio of the number of photons produced by the source and directed at a particular illumination point and the number of photons detected from a given collection point, is a function, *inter alia*, of the power of the light source and the distance from the illumination point at which the light is collected. The intensity of the source may be adjusted so that the flux of photons reaching the detector is optimized for the characteristics of the detector. In a preferred embodiment, in which TD imaging of small mammals is performed using a TCSPCD detection system, it is generally required that the probability of detecting a photon for each illumination light pulse be approximately

1% in order to avoid distortions caused by electronics dead-time losses in the temporal profile being measured as is well known by a person skilled in the art. The illumination duration (which is provided by the number of light pulses) directed at a given point on the mammal may vary in order to provide a sufficient number of detected photons to produce an adequate signal and yet keep the duration as short as possible to reduce the acquisition time. In accordance with the example of small animal imaging and using a light source emitting pulses at a frequency of 80MHz, the power of the beam should be adjusted so that approximately 8×10^5 photons per second are detected. It will be appreciated that the selection of the appropriate frequency is based on, among other factors, the characteristics of the optical components, of the detector, of the tissue to be imaged and of the type of optical data that is desired.

[0040] The TPSF may also be generated by using a time gated intensified charged coupled device (ICCD). This type of detector can provide spatial resolution enabling simultaneous detection of optical signals emanating from different collection points. Furthermore when a source generating two or more wavelengths is used, the light collected at any given collection points can be divided into constituent wavelengths to produce two or more beams which can be directed to different detection positions of the ICCD. However, since the sensitivity of an ICCD is less than that of a TCSPCD, the intensity of the source should be adjusted accordingly while remaining below levels that could cause tissue damage.

[0041] In view of the low intensity levels of the source, especially in the case where TCSPCDs are used, and the

high sensitivity of the detectors, the system and the mammal are placed in an enclosure such as a box 50. The box is preferably light tight to prevent any stray light from interfering with the measurements. The interior of the box can be accessed through door 56.

[0042] In order to construct an image of a region of interest (ROI) within the mammal, optical signals are obtained from a plurality of illumination/detection points within the ROI. The configuration of the illumination/detection points may vary depending of the type of image to be reconstructed. As will be explained below topographic and tomographic images can be generated with the system and method of the present invention and both require different illumination/detection configurations.

[0043] The position of the collection points relative to the illumination points is determined prior to the start of the acquisition and is a function of the desired depth of imaging in the region of interest. For planes of imaging that are close to the surface of the skin, the collection points are located near the illumination points since the deeper a photon travels, the lower is the probability of that photon being re-emitted near the illumination points, and conversely.

[0044] Thus in order to acquire topographic images collection points are maintained at a fixed distance from illumination points so as to gather information from substantially the same depth across the ROI. The mirror galvanometers comprised in the illuminating and the collecting optic may be synchronized to achieve rapid

scanning with a constant illumination/detection points distance.

[0045] Tomographic data is obtained when the illumination and collection points are permuted so as to generate a plurality of illumination to collection points distances thereby obtaining information from different depths. By treating the data in an appropriate manner, depth resolved tomographic images (which is a form of tomography) may be constructed. For tomographic optical data acquisition the two mirror galvanometers 24 and 26 are preferably controlled independently in order to obtain multi-perspective data. Thus while the mirror galvanometer in the illumination optic directs the light at a desired illumination point, the mirror galvanometer in the collection optic may be programmed to sample light at a plurality of different collection points.

[0046] In one embodiment, the illumination and collection optics are mounted on a movable gantry system 52 which turns around the animal (Fig. 5). In this particular embodiment, the tray preferably exhibits an "I" shape. This particular shape facilitates data acquisition as the illumination and collection optics is rotated around the region of interest (for example: torso region) while allowing the animal to be comfortably supported. With this configuration, angular displacements of an amplitude substantially equals to 360 degrees are possible. In a further embodiment, the mammal may be rotated instead of the gantry. The mammal may be maintain on the tray by attaching its legs to the tray. Rotation around the cranio-caudal axis of the body by 360° is possible. This design configuration can reduce weight, volume and complexity compared to the moveable gantry system.

[0047] In yet another embodiment the mammal can be rotated along the cranio-caudal axis while sitting. The animal is positioned on the stage such that the region of interest is kept straight by softly supporting its head. These designs allow the animal to be scanned over almost 360°. It will be appreciated that the optics may be modified to adapt it to the different tomographic configurations. For example, mirror 54 in the "sitting" configuration provides a convenient way of directionally propagating the light.

[0048] The acquisition of optical data at a plurality of angles around the animal may result in appreciable variations in the distance between the surface of the ROI and the collection optics because of the irregular contour of the animal. Accordingly image reconstruction may be improved by the use of an auto-focus system and by obtaining a profile of the scanned regions.

[0049] While the imaging of biological tissue can rely on the natural optical properties of the endogenous molecules for providing optical contrast, exogenous molecules may be introduced in the tissue to provide additional contrast. In this respect, exogenous chromophores as well as fluorophores may be used. Furthermore the biodistribution of such contrast agents can be followed using the method and system of the present invention. In one advantageous embodiment the biodistribution can be followed over time thereby producing pharmacokinetics data.

[0050] The optics as well as the source can be arranged to illuminate and detect light at one or more wavelengths as is described *supra*. This property can be exploited to

follow the pharmacokinetics of two or more fluorophores and/or chromophores.

[0051] The embodiment(s) of the invention described above is(are) intended to be exemplary only. The scope of the invention is therefore intended to be limited solely by the scope of the appended claims.

I/WE CLAIM:

1. A method for collecting optical data for use in time resolved optical imaging of a biological tissue, the method comprising:

   i) directionally propagating through free-space optics a pulsed light beam of a selected intensity to illuminate at one or more wavelength a plurality of predetermined illumination points in a region of interest of the biological tissue;

   ii) selectively collecting, through free-space optics, light emanating from a plurality of predetermined collection points;

   iii) directionally propagating through free-space optics the collected light towards a detector;

   iv) measuring, at one or more wavelength, the collected light at the detector to produce a time resolved optical signal for one or more illumination points/collection points configuration; and

   wherein light emanating from points other than the predetermined collection points is optically excluded from detection.

2. The method as claimed in claim 1 wherein the time resolved optical imaging is time domain (TD) imaging and wherein the time resolved optical signal is detected such as to generate information related to a time point spread function (TPSF).

3. The method as claimed in claim 2 wherein the step of measuring comprises detecting the collected light using single photon counting.

4. The method as claimed in claim 3 wherein each illumination point is illuminated by a plurality of pulses.

5. The method as claimed in claim 4 wherein the step of illuminating comprises adjusting the intensity of the light beam such as to avoid distortions caused by electronics dead-time losses.

6. The method as claimed in claim 5 wherein the intensity is adjusted by varying the source intensity.

7. The method as claimed in claim 6 wherein the intensity is adjusted with filters.

8. The method as claimed in claim 1 wherein the optical signal is detected at two or more wavelengths simultaneously.

9. The method as claimed in claim 1 wherein the illumination points are illuminated in a raster scan fashion.

10. The method as claimed in claim 1 wherein the collection points are located at a fixed distance from the illumination points to provide optical signal for topographic imaging .

11. The method as claimed in claim 10 wherein the distance is about 3mm.

12. The method as claimed in claim 1 wherein two or more collection points are collected for each illumination point to provide optical data for tomographic imaging.

13. The method as claimed in claim 12 wherein at least two of the 2 or more collection points are collected simultaneously.

14. The method as claimed in claim 1 wherein detection is effected at a wavelength different from that of illumination.

15. The method as claimed in claim 1 wherein the biological tissue comprises one or more fluorophores and wherein the detection wavelength corresponds to an emission wavelength of the one or more fluorophores and the illumination wavelength corresponds to an excitation wavelength of the one or more fluorophores.

16. The method as claimed in claim 15 wherein both the excitation and emission wavelength are detected.

17. The method as claimed in claim 2 wherein the TPSF is integrated to provide attenuation measurement.

18. The method according to claim 1 wherein the biological tissue is comprised in a mammal.

19. The method as claimed in claim 18 wherein optical data from a plurality of regions of interest are collected during a single session.

20. The method as claimed in claim 19 wherein the plurality of regions of interest comprises a whole body of a mammal.

21. A system for collecting optical data for use in time resolved optical imaging of a biological tissue, the system comprising:

i) one or more pulsed light source of selected intensity for providing a light beam at one or more wavelengths;

ii) illuminating optic components for directionally propagating the beam through free space optics such that a region of interest of the biological tissue is illuminated at a plurality of illumination points thereby injecting light into the tissue;

iii) collecting optic components for collecting through free space optics light re-emitted at a plurality of predetermined collection points in the region of interest such that light emanating from points other than the predetermined collection points is optically excluded from detection, and for directionally propagating, through free space optics, the collected light; and

iv) a time correlated detector for detecting the collected light.

22. The system as claimed in claim 21 wherein the one or more light sources are variable intensity light sources.

23. The system as claimed in claim 22 wherein the variable intensity light sources are lasers.

24. The system as claimed in claim 23 wherein the illuminating optic components comprise at least one moveable mirror for directing the beam to the plurality of illumination points.

25. The system as claimed in claim 24 wherein the moveable mirror is a mirror galvanometer

26. The system as claimed in claim 25 further comprising a thin angled mirror located optically downstream of the mirror galvanometer.

27. The system as claimed in claim 26 wherein a lens is positioned between the mirror galvanometer and the thin angled mirror and optically coupled therewith to provide a telecentric imaging configuration.

28. The system as claimed in claim 27 wherein the collecting optic components comprise a lens located above the region of interest and having a focal point coincident with the collection point.

29. The system as claimed in claim 28 wherein the collecting optic components further comprise a mirror galvanometer for directing the collected light to the detector.

30. The system as claimed in claim 29 wherein the mirror galvanometers of the illumination optic and collection optic are synchronized so as to provide a fixed distance between the illumination points and respective detection points.

31. The system as claimed in claim 30 wherein the illumination optics, the detection optics and the source are part of a gantry that can be rotated around the biological tissue.

32. The system as claimed in claim 21 further comprising a translational stage for moving a tray in a plane perpendicular to the illuminating beam.

33. The system as claimed in claim 32 wherein the tray is for supporting a mammal comprising the biological tissue.

34. The system as claimed in claim 33 wherein the tray is heated.

35. The system as claimed in claim 21 wherein the detector is a single photon counting detector.

36. The system as claimed in claim 21 wherein the detector is a time gated ICCD.

37. The system as claimed in any one of claims 21-36 wherein the mammal, the optical components and the detector are contained in an enclosure.

38. The system as claimed in claim 37 wherein the enclosure is light tight.

ABSTRACT OF THE DISCLOSURE

There is provided a method and system for collecting optical data for use in time resolved optical imaging wherein light is directionally propagated through free-space optics to impinge on a plurality of illumination at the surface of a biological tissue such as that comprised in small mammals. Light re-emitted from the tissue is collected and directionally propagated through free space optics towards a detector to produce time resolved optical signals useful for optical image reconstructions.

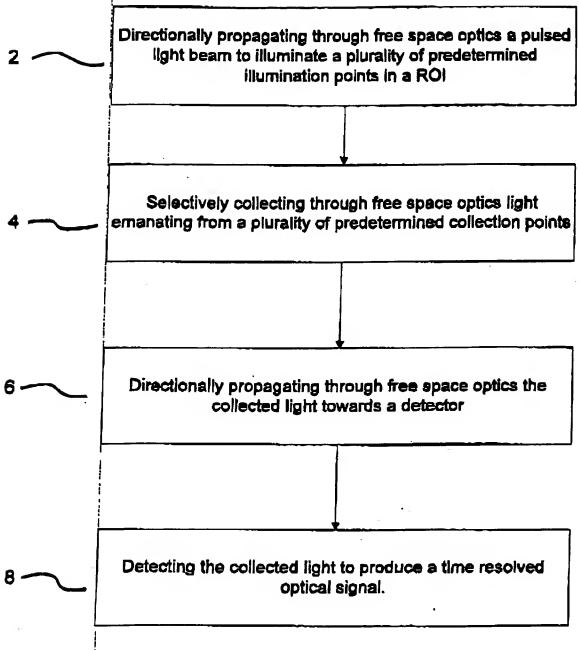
2
Directionally propagating through free space optics a pulsed light beam to illuminate a plurality of predetermined illumination points in a ROI
4
Selectively collecting through free space optics light emanating from a plurality of predetermined collection points
6
Directionally propagating through free space optics the collected light towards a detector
8
Detecting the collected light to produce a time resolved optical signal.

Fig. 1

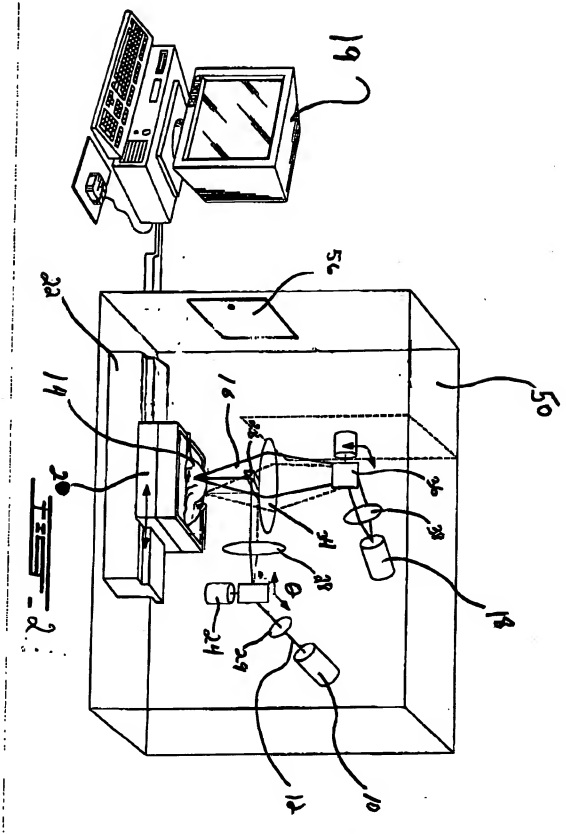

FIG. 2

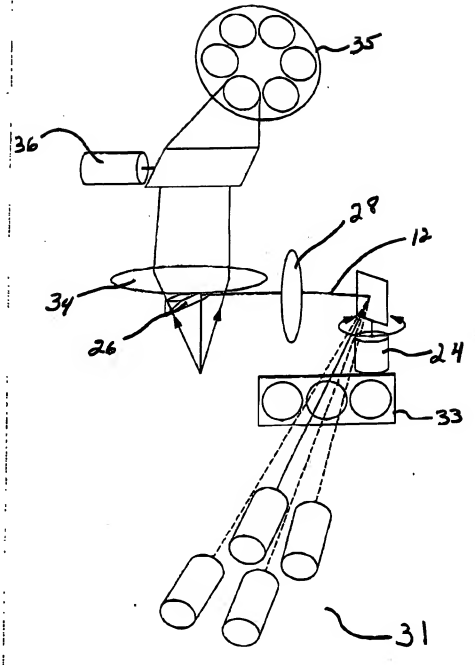

Fig-3

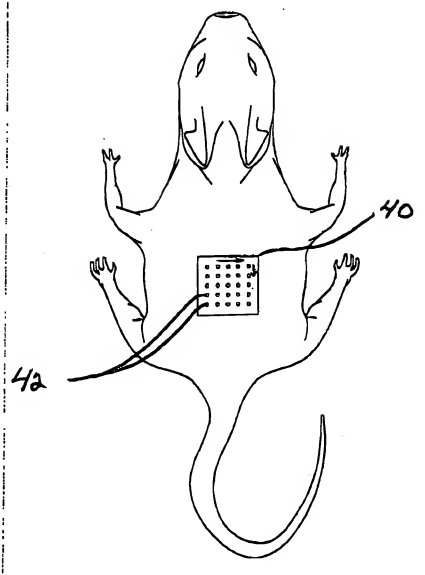

Fig. 4

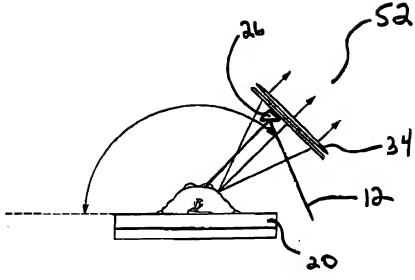

FIG- 5

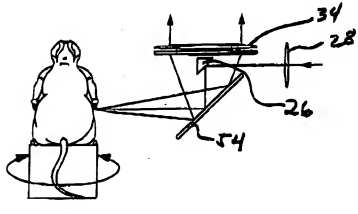

FIG- 6

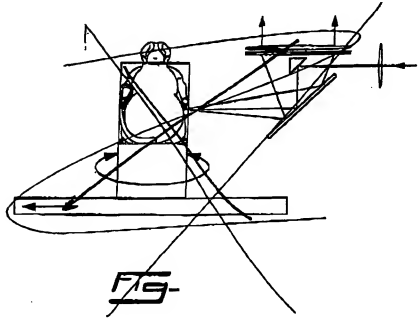

FIG-